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- Published:
— *with international search report*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **FEED FOR AQUATIC SPECIES**

(57) Abstract: The invention relates to a feed for aquatic species and crustaceans, in particular marine species and fry thereof. The feed described comprises proteins, lipids and additional, optional components, and is characterized in that the lipids are one or more marine oils and/or vegetable oils treated by urea and/or other amines or amides, to prevent degradation due to oxidation (oxidative stress).

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Feed for aquatic species

This invention relates to a feed for aquatic species including fry.

5 A problem for the aquaculture industry has been degradation and low quality of the fat components in the feed due to oxidation. This problem has previously been focused on farming of salmonids.

10 When marine fat, which is the main fat source in fish feed, reacts with oxygen, firstly primary oxidation products like peroxides are formed. The level of primary oxidation products is normally analysed as peroxide value. Peroxides from polyunsaturated fat are unstable and easily degraded, resulting in transformation to secondary oxidation products.

15 Secondary oxidation products are a complex group of compounds including aldehydes and ketones. To quantify the amount of secondary oxidation products the anisidine value is measured. The anisidine value is the intensity of a colour that develops during reaction between the reagent anisidine and aldehydes in the fat. The anisidine value is
20 given without denomination.

The level of oxidation in a fat is often given as totox-value. The totox-value is two times the peroxide value added with the anisidine value.

25 In feed for salmonids oils having low totox-values should be used In order to secure optimal growth for the fish.

In Norwegian Patent No. 309795 (corresponding to International patent application No. WO 00/01249) in the name of the present Applicant, a method for stabilising vegetable
30 and animal oils by treating the oil by urea and optionally one or more antioxidants, and a feed for salmonids comprising inter alia oils treated by the said method are disclosed. Norwegian Patent No. 311041 (corresponding to International patent application No. WO 01/46355) also in the name of the present Applicant, further discloses a method for stabilising similar oils by treatment of one or more other amines or amides. In NO-
35 309795 and NO-311041 it is reported that by producing astaxanthin-containing fish feed for salmonids with oil produced according to said methods, the degradation of astaxanthin during extrusion of feed pellets is considerably reduced.

At the time when NO-309795 and NO-311041 were filed, the aquaculture industry was focused on farming of salmonids. The aim of the said inventions was to reduce the degradation of astaxanthin and similar pigments, the most expensive components of fish feed. Now, however, other marine species have also become an object of the aquaculture industry. These species, like cod and halibut, face the fish farming industry with new challenges. Feed for these species normally does not contain significant amount of carotenoids like astaxanthin or canthaxanthin. However, so far it seems as if these species are more difficult to raise with optimum health and growth. One problem is related to low survival and low growth rate of fry and small fish.

In production of such marine species breeding of fry is a problem. The mortality of the fry is very high. In addition the rate of weight gain, especially of small fish, is lower than desired. One reason for this is that the fish is sensitive to oxidative stress. When the fish is fed a highly oxidized feed, the oxidative stress becomes high.

Thus, it is desirable to have access to a feed for the marine species and fry thereof where the oxidative stress of the feed is minimal.

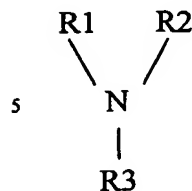
The present invention is in a surprising manner able to provide a feed more suitable for marine species and fry in particular, than previously known feed.

The essential features of the present invention are given in the accompanying claims.

A person skilled in the art will realize that a feed according to the present invention will be useful for all aquatic species and crustaceans.

A feed for aquatic species including fry, containing lipids that comprises one or more marine oils and/or vegetable oils treated by urea and/or other amines or amides and optionally one or more antioxidants is disclosed in the present invention.

The above-mentioned amines and amides are defined by the general formula:



10 wherein each of R1, R2 and R3 are independently chosen from H, C₁-C₁₀-alkyl, C₂-C₁₀-alkenyl, or RC(O)- or RN(H)-C(O)-C(O)- where R is H, C₁-C₁₀-alkyl or C₂-C₁₀-alkenyl, or RN(H)-C(O)- where R' is C₁-C₁₀-alkyl, C₂-C₁₀-alkenyl, or H if at least two of R1, R2 and R3 are different from H.

15 According to one embodiment of the invention oils are heated in presence of urea and/or other amines or amides, preferably above the melting point of the nitrogen containing compound(s) and preferably kept at this temperature for a time period of approximately 20-30 minutes, and one or more antioxidants may be added. According to another embodiment of the invention the oil is reacted with an aqueous mixture of 0.1-50% of urea and/or other amines or amides, preferably 0.5-5% of urea and/or other amines or
20 amides. The preferred antioxidants are tocopherol and/or ascorbic acid, and tocopherol and/or ascorbyl palmitate.

The invention also discloses the use of one or more marine oil and/or vegetable oil treated by urea and/or other amines or amides and optionally one or more antioxidants
25 for production of a feed for marine species including fry, which reduces degradation of the feed with regard to oxidation.

A preferred feature of this invention is that the oil is treated with urea and/or other amines or amides and added to the feed before or after extrusion. The oil is treated
30 either by heating in the presence of the nitrogen containing compound(s), or by reacting with an aqueous mixture of nitrogen containing compound(s). Preferably the urea and/or amines and amides are removed from the oil, e.g. by filtration, before the oil is added to the feed.

35 Another feature of the invention is that the nitrogen containing compound(s) is added directly to the feed mixture, either in an aqueous phase or in solid form.

The nitrogen containing compound(s) may however be added in a number of ways and not only directly to an oil. By production of a feed urea and/or other amines or amides can be added for instance during the extruding, by vacuum coating, spray coating and
5 by oil bath. The nitrogen containing compound(s) can also be added in the water phase or in solid form.

A preferred nitrogen-containing compound according to this invention is urea.

10 Among the marine species, cod and halibut are among the species that are of interest in the aqua culture industry.

In the following the invention will be further explained by examples and a figure. The examples are just meant to be illustrative and shall not be considered as limiting.

15

Figure 1 shows secondary oxidation products expressed in anisidine number for omega oils treated with fish meal. Results for oils pre-treated with urea and oils not pre-treated with urea are shown.

20

Example 1

Fish feed is extruded according to a recipe identical to Example 7 of NO-309795. The extruded and dried feed pellets are analysed by using Electron Spin Resonance. This is a
25 method for elucidating the presence and relative concentration for free radicals. Free radicals are associated with ongoing oxidation; i.e. a high level of free radicals in a sample is associated with a high oxidative stress of that sample.

30

35

Results:

	ESR (pp-amp)
Fish meal and wheat flour	2.0
Fish meal and wheat flour plus fish oil	1.7
Fish meal and wheat flour plus fish oil. Heated at 125°C for 5 min.	2.8
Pellets, commercial formula	2.8
Pellets, urea-treated oil in extruder	1.7

The ESR-results (pp-amp) give an indication of the concentration of free radicals in the sample. A high numerical number indicate a high level of free radicals. The mixture of fish meal and wheat flour used for the fish feed gave a numerical result of 2.0 on this relative scale. When fish oil was added, the value was 1.7. After heating a sample of this mixture for 5 minutes at 125°C, simulating extrusion, the value had increased to 2.8. This was identical to the value after extrusion and drying for feed pellets produced from the same mixture of meal, flour and oil. However, when the oil was substituted with an oil treated with urea according to the present invention, the value was 1.7 also after extrusion. Surprisingly, the extrusion process had not resulted in an increase in free radical activity of the dried pellets.

This shows that the present invention gives a feed that reduces the oxidative stress of a feed composition.

The positive effect of the invention in showing a reduced oxidation of fish feed can be shown by storing samples of feed in an atmosphere of oxygen, and under increased pressure, with continuous recording of oxygen consumption. The oxygen consumption is lower when an oil according to the present invention is used, compared to feed produced with untreated oil.

Example 2

A South American fish oil intended for fish feed ("omega oil") mixed with 20 % (low temperature) LT fishmeal was heated to 60°C with stirring. The oil was left for 30
5 minutes.

Initially the oils treated in this way (control) had an anisidine number of 20.

For the aliquots of this oil treated according to NO-309795 (i.e.; heated with urea,
10 cooled, and filtrating off the urea) the initial anisidine numbers were 2.

As can be seen in Fig. 1, the anisidine number of the control is rapidly reduced. It is assumed that this is due to a reaction between amino acids/protein compounds and carbonyl compounds (i.e. secondary oxidation products), and that a similar reaction
15 takes place during feed production. It is further assumed that this is unfavourable for the value of the feed, which again indicates that commonly manufactured feed gives disadvantageous effects. Commercially available feed makes the proteins less bioavailable for the fish and/or they decompose in the intestine of the fish and thereby secondary oxidation products are released.

20 Wild fish do not experience the problems mentioned above, as wild fish do not eat oxidized feed.

By the feed according to the present invention, the oxidation problems nearly are
25 solved. The present invention provides a feed of improved quality.

The main object of the present invention is to provide a feed for marine species, included fry, where the oxidative stress of the feed due to fat oxidation is minimal. By treating the marine and/or vegetable oils in the feed by urea and/or other amines or
30 amides as given above, this object is fulfilled. The feed according to the present invention is due to its minimal oxidative stress particularly suitable for marine species and fry. It is assumed that oxidative stress is one reason for the health problems, low survival and growth rate of fry and small fish. By feeding a feed according to the present invention to these species, fish of improved quality with regard to health,
35 growth and survival are obtained. The possibility by using a feed with a minimal oxidative stress given by the present invention is a very important contribution within farming of marine species in the aquaculture industry.

Furthermore, heart rupture is a survival/health problem for salmonids and other farmed fish species. This particular health problem is assumed occur due to oxidative stress in the feed. Thus, using the feed according to the present invention is suitable also for salmonids in order to reduce the heart rupture problems of the fish.

By applying the method for stabilising vegetable and marine oils as disclosed in NO-309795 and NO-311041, lipids causing oxidative stress in feed for marine species are obtained. In this way a feed for marine species and fry in particular, that causes less oxidative stress to the fish is obtained. This feed is also suitable for salmonids, particularly for reducing the occurrence of heart rupture.

The present invention implies an improved solution to the breeding problem of fry of marine species as described above. A satisfactory solution to this problem has previously not been found.

The person skilled in the art will realise that similar problems related to non-optimal growth and health due to oxidative stress from the feed will be relevant to all aquatic species, not only fish.

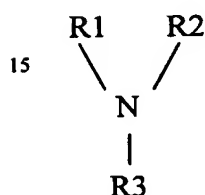
C l a i m s

1.

5 Feed for marine species including fry, comprising 25-70 % by weight of proteins, 5-60 % by weight of lipids, 0-40 % by weight of carbohydrates, and 0-15 % by weight of one or more additional components; such as fillers, adhesives, preservatives, vitamins and minerals,

characterized in that some or all the lipids are one or more marine oils
 10 and/or vegetable oils treated by urea and/or other amines or amides, and optionally one or more antioxidants,

wherein the said amines and amides are defined by the general formula (I):



20 wherein each of R1, R2 and R3 are independently chosen from H, C₁-C₁₀-alkyl, C₂-C₁₀-alkenyl, or RC(O)- or RN(H)-C(O)-C(O)- where R is H, C₁-C₁₀-alkyl or C₂-C₁₀-alkenyl, or R'N(H)-C(O)- where R' is C₁-C₁₀-alkyl, C₂-C₁₀-alkenyl, or H if at least two of R1, R2 and R3 are different from H.

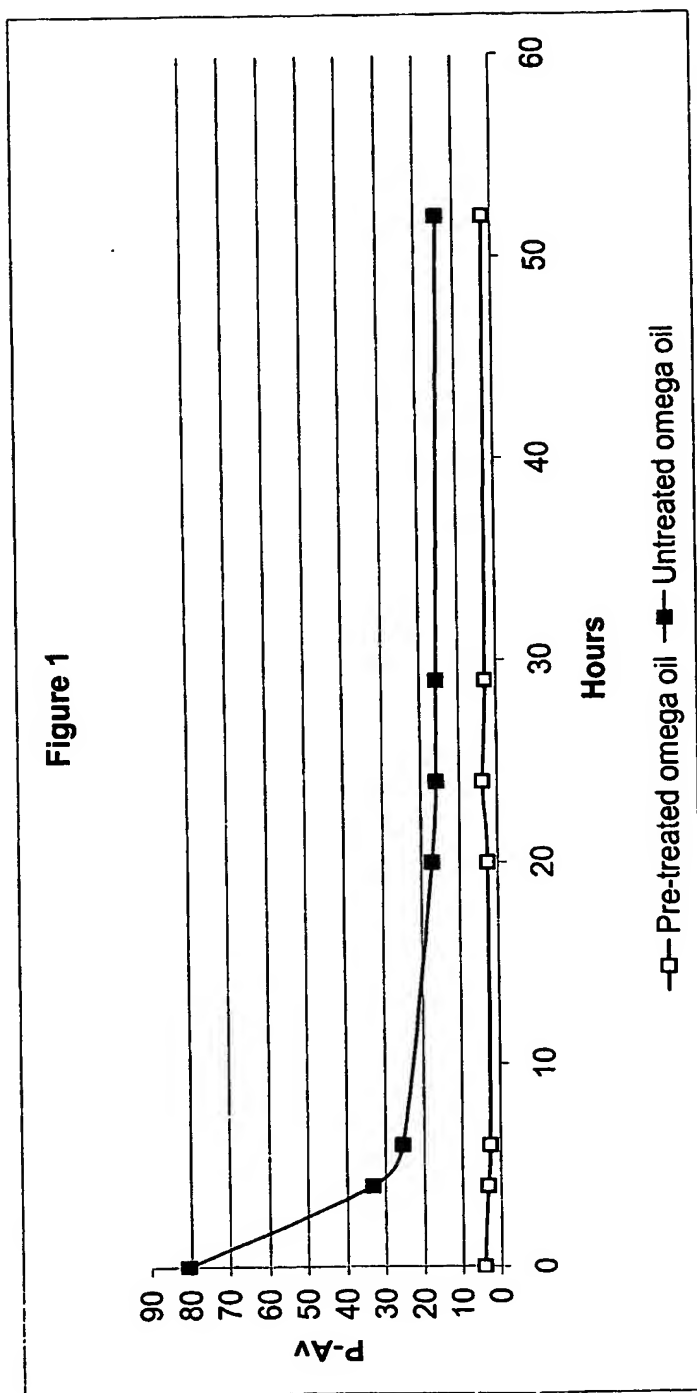
25 2. Feed according to claim 1, characterized in that the urea and/or amines or amides are substantially removed from the oil after treatment.

3. Use of one or more marine oil and/or vegetable oil treated by urea and/or other amines or amides of formula (I), and optionally one or more antioxidants for production
 30 of a feed for marine species including fry, which reduces degradation of the feed.

4. Use of one or more marine oil and/or vegetable oil treated by urea and/or other amines or amides of formula (I), and optionally one or more antioxidants for production
 35 of a feed for marine species including fry for improving the health condition, and survival and growth rate of the fish.

5. Feed according to claim 1 or use for production of feed according to claims 2, 3 or 4, for all aquatic species and crustaceans.

6. Use of one or more marine oil and/or vegetable oil treated by urea and/or other
5 amines or amides of formula (I), and optionally one or more antioxidants for production of a feed for marine species and salmonids for reducing occurrence of heart rupture of the fish.



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/NO 03/00084

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A23K 1/00, C11B 5/00, A23D 9/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A23K, C11B, A23D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CHEM. ABS DATA, EPO-INTERNAL, WPI DATA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 0001249 A1 (NORSK HYDRO ASA), 13 January 2000 (13.01.00) --	1-6
X	WO 0146355 A1 (NORSK HYDRO ASA), 28 June 2001 (28.06.01) --	1-6
X	Daskalov H. et al; "Influence of oxidized lipids in diets on the development of rainbow trout fry syndrome"; Journal of Fish Diseases, 23, 1 (January 2000), pages 7-14. --	1-6

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

16 June 2003

Date of mailing of the international search report

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Olsen R.E. et al; "The influence of temperature, dietary polyunsaturated fatty acids, α -tocopherol and spermine on fatty acid composition and indices of oxidative stress in juvenile Arctic char, <i>Salvelinus alpinus</i> (L.)"; Fish Physiology and Biochemistry, 20, 1 (January 1999), pages 13-29. --	1-6
X	Waagbø, R.; "The impact of nutritional factors on the immune system in Atlantic salmon, <i>Salmo salar</i> L.: a review"; Aquaculture and fisheries management, 25 (1994), pages 175-197. --	1-6
A	WO 9612415 A1 (NORSK HYDRO A.S), 2 May 1996 (02.05.96) -- -----	1-6

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/NO03/00084

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: **1-6**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Present claims 1 - 6 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and / or disclosure within the meaning of Article 5 PCT is to be found for only a very small proportion of the compounds to which the present claims relate. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search has been carried out only for those parts of the claims which appear to be supported and disclosed, namely those parts related to urea and the amines and amides in WO0146355 (page 5, Table 1), namely: allylurea, hexylamine, N-ethyl-diisopropylamine, N,N'-dimethylurea, Oxamide and butyramide.

INTERNATIONAL SEARCH REPORT
Information on patent family members

02/06/03

International Application No.

PCT/NO 03/00084

Patent document cited in search report			Publication date	Patent family member(s)		Publication date
WO	0001249	A1	13/01/00	AU	4399999 A	24/01/00
				CA	2336272 A	13/01/00
				CN	1307453 T	08/08/01
				EP	1091657 A,B	18/04/01
				JP	2002519479 T	02/07/02
				NO	309795 B	02/04/01
				NO	983050 A	03/01/00
				ZA	200007556 A	15/03/02
WO	0146355	A1	28/06/01	AU	2238601 A	03/07/01
				CA	2394622 A	28/06/01
				EP	1240285 A	18/09/02
				NO	311041 B	01/10/01
				NO	996411 A	25/06/01
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				CA	2203106 A	02/05/96
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				CN	1169659 A	07/01/98
				DE	69508420 D	00/00/00
				DK	786946 T	11/10/99
				EP	0786946 A,B	06/08/97
				ES	2131866 T	01/08/99
				GR	3030527 T	29/10/99
				JP	10508197 T	18/08/98
				NO	944028 D	00/00/00
				US	5874118 A	23/02/99